Analysis of Perfluoroalkyl Carboxylic Acids in Composite Dietary Samples by Gas Chromatography/Mass Spectrometry with Electron Capture Negative Ionization (ガスクロマトグラフィー負化学イオン化質量分析による食事中のフッ素化カルボン酸の分析)

（論文内容の要旨）

有機フッ素化合物のペルフルオロアルキルカルボン酸類 [Perfluoroalkyl carboxylic acids ; PFCAs , $CF_3(CF_2)_nCOOH$]は撥水加工剤製造等に使用されてきた化学物質であるが、難分解性であることから広範囲の環境汚染が懸念されており、環境汚染の実態把握が必要である。本研究では、ガスクロマトグラフィー質量分析(Gas Chromatography/Mass Spectrometry; GC-MS)を用い、化学修飾した測定対象を負化学イオン化(Electron capture negative ionization; ECNI)法で測定することにより、簡易かつ高感度な PFCAs 分析法を開発した。その分析法を用いて現在まで報告が限られている食事試料中の PFCAs を測定、摂取量について日本・中国・韓国の東アジア 3 か国で報告した。

PFCAs の分析は現在まで液体クロマトグラフィー/タンデム質量分析や GC-MS の電子イオン化(Electron impact ionization; EI)法が多く用いられているが、いずれの方法でも食事等の複雑な夾雑物を持つ試料の微量分析は難しいとされてきた。本研究では食品から抽出した PFCAs を臭化ベンジルを用いてベンジル基で化学修飾し、得られた PFCAs ベンジルエステル[$CF_3(CF_2)_n$-COO-C_7H_7]を GC-MS の ECNI 法で測定した。その結果、マススペクトル上に非常に強い PFCAs アニオン($CF_3(CF_2)_nCOO^-$)に対応するフラグメントを得ることができた。この方法でのシグナルノイズ比 3 で設定された機器検出限界値は 0.003-0.007pg となり、現行の EI 法の機器検出限界値より 50 から 285 分の一に相当する低い値を得た。1g の食事試料を使用した場合の分析法検出限界値も、操作ブランクを考慮しても 2-10pg/g と十分に低い値であった。なお抽出法は凍結乾燥や固相抽出等の煩雑な処理を避けるため、イオンペア抽出法を用いた。

上記の分析法を用いて炭素鎖 8 から炭素鎖 14 の PFCAs を対象に京都大学生体試料バンクに保管されている日本（京都・北海道・沖縄）、中国（北京）、韓国（ソウル）の 5 地点の各 1990 年代、2000 年代の食事試料 60 検体を分析した。1990 年代における摂取量は中央値で京都 46.7ng/day、北海道 79.3ng/day、沖縄 55.5ng/day、北京 66.6ng/day、ソウル 40.4ng/day であった。一方、2000 年代における摂取量は京都 56.6ng/day、北海道 57.8ng/day、沖縄 86.8ng/day、北京 68.1ng/day、ソウル 172.8ng/day であった。このようにソウルでは 1990 年代に比べて 2000 年代で約 4 倍の上昇がみられた。

Analysis of perfluoroalkyl carboxylic acids in composite dietary samples by gas chromatography/mass spectrometry with electron capture negative ionization

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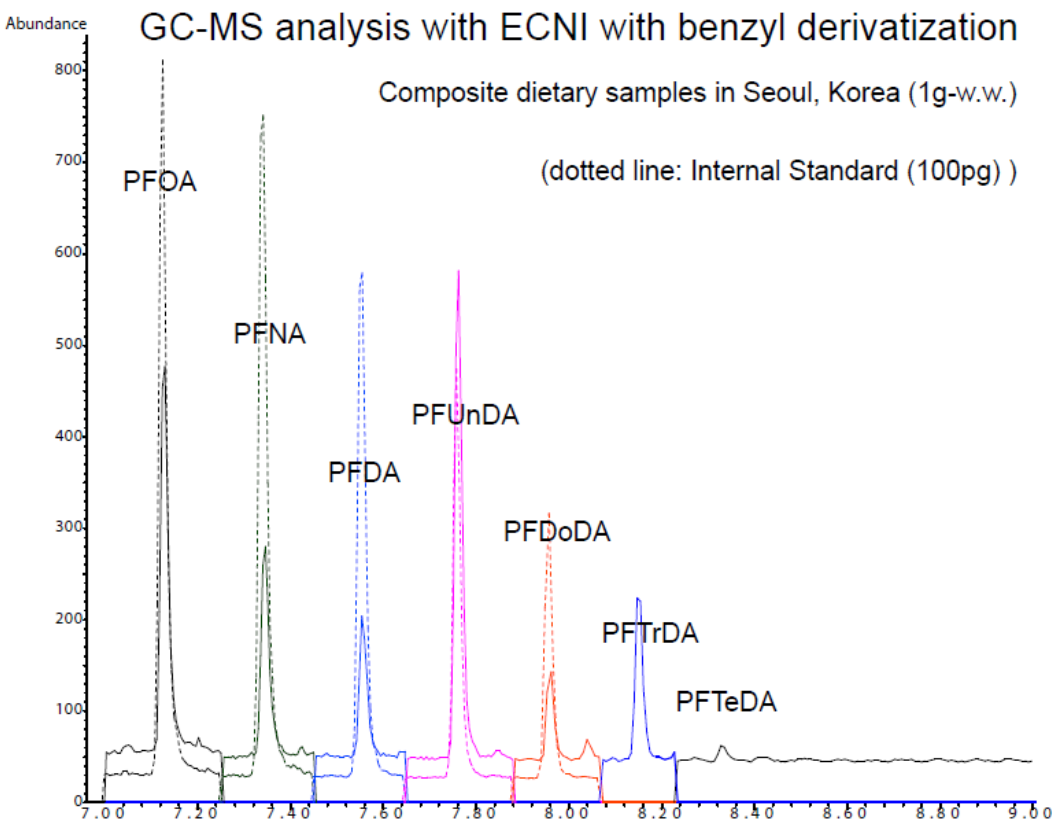

Abstract

In this study, a gas chromatography-mass spectrometry and electron-capture negative ionization (ECNI) method was developed to quantify perfluorinated carboxylic acids (PFCAs) in composite dietary samples. Benzyl esterification was used for pre-treatment before PFCAs analysis. This stabilized the benzyl radical leaving group preferentially, and gave carboxylic anions of the PFCAs with ECNI. The method had a low detection limit (0.3–10 pg g^{-1}) and good recoveries (98–90 %) for PFCAs with eight to 14 carbon atoms (C8 to C14). The method was applied to 24-h dietary samples from subjects in Japan (Hokkaido, Kyoto, and Okinawa; 1992 and 2009), Korea (Seoul; 1994 and 2007), and China (Beijing; 1993 and 2009). The levels of the PFCAs were between 39 ng day^{-1} and 169 ng day^{-1} (per kilogram of body weight) in Korea, 58 ng day^{-1} and 71 ng day^{-1} in China, and 56 ng day^{-1} and 67 ng day^{-1} in Japan. Between the two sampling years, the total levels of PFCAs (C8 to C14) increased significantly (p<0.05). The interaction between the sampling location in Korea and year was significant (p<0.05).

Abbreviations

PFCAs: perfluorinated carboxylic acids

PFOA: perfluorooctanoic acid

PFNA: perfluorononanoic acid

PFDA: perfluorodecanoic acid

PFUnDA: perfluoroundecanoic acid

PFDoDA: perfluorododecanoic acid

PFTrDA: perfluorotridecanoic acid

PFTeDA: perfluorotetradecanoic acid

IDLs: instrumental detection limits

MDLs: method detection limits

GC/MS: gas chromatography and mass spectrometry

ECNI: electron-capture negative ionization

1. Introduction

Perfluorochemicals such as perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are environmental contaminants of public health concern because of their persistence and bioaccumulation in the environment. They have been detected in human serum and breast milk samples in many areas.[1] However, the exposure routes are not well characterized. The 3M Company was a major manufacture of PFOS, but phased out its production in 2002.[2] Since then, concern has shifted from PFOS to PFOA. However, in Japan, perfluorinated carboxylic acid (PFCA) emissions contain perfluorononanoic acid (PFNA) and perfluoroundecanoic acid (PFUnDA) in addition to PFOA.[3] In 2000, 25 and 7 t (1 t = 1000 kg) of PFNA and PFUnDA were emitted, respectively.[3] In an earlier study, we detected PFNA and PFUnDA at concentrations comparable to PFOA in serum samples from Japanese, Korean, and Vietnamese adults[4]. The levels of these chemicals have continued to increase in humans, even after production PFOS was halted in 2002,[4] but the exposure routes that are contributing to these increases are still unknown.

Although dietary intake is thought to be a major route of exposure for PFCAs,[5] there is little data for long chain PFCA levels in food items because of analytical difficulties with method development in liquid chromatography and mass spectrometry (LC/MS).[6] Quantitative analyses of PFCAs at low pg g^{-1} concentrations in complex matrices such as food require rigorous and complicated clean-up procedures to eliminate matrix effects.[7] Alternatively, gas chromatography-mass spectrometry (GC/MS) can be used as the matrix suppression effect caused by co-eluting material is relatively low compared with LC/MS.[8] Electron-capture negative ionization (ECNI) reportedly improves the detection limits of PFCA anilides,[9] and could be coupled with

GC/MS.

The aim of the present study was to develop a simple but sensitive quantification method for PFCAs in foods. GC/MS was coupled with ECNI for PFCAs analysis. ECNI improved the sensitivity for PFCAs benzyl esters compared with electron impact ionization (EI). PFCAs in composite dietary samples were quantified by this method, and historical trends in the dietary intake of PFCAs in three Asian countries were analyzed.

2. Material and methods

2.1. Chemicals

Acetone (LC-MS grade), sodium carbonate (>99.5%) and distilled water (LC-MS grade) were obtained from Kanto Chemicals (Tokyo, Japan). Benzyl bromide, tetrabutylammonium hydrogen sulfate, 11H-perfluoroundecanoic acid and methyl tertiary-butyl ether (MTBE, HPLC grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). A mixture of $^{13}C_2$-labeled perfluorohexanoic acid, $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled perfluorododecanoic acid (PFDoDA) was obtained from Wellington Labs (Guelph, ON, Canada). $^{13}C_{12}$- 2,3,3',5,5'-pentachlorobiphenyl (CB-111) was obtained from Cambridge Isotope Laboratories (Andover, MA)

2.2. Sample collection

Diet samples from the Kyoto University Human Specimen Bank[10, 11] were used for the evaluation. At the time of collection, participants were requested to donate duplicate samples of all food and drink items that they consumed over a 24-h period. These samples are referred to as duplicate 24-h diet samples. Two hundred duplicate 24-h diet

samples were collected from the following locations: Hokkaido (Japan) in 1992 and 1995, Okinawa (Japan) in 1992 and 1995, Kyoto (Japan) in 1996 and 1997, Beijing (China) in 1993 and 2009, and Seoul (Korea) in 1994 and 2007.[10, 12] In addition, 100 duplicate 24-h diet samples (i.e. a typical day's worth of food and drink) were purchased by volunteers from markets in Kyoto, Okinawa, and Hokkaido in 2009. The study populations were the same as those in earlier studies.[13, 14] This gave a total of 300 duplicate 24-h diet samples. All food and drink samples in each duplicate sample were mixed together and homogenized. Then the 300 homogenized diet samples were combined into 60 groups (150 g), referred to as the composite dietary samples, each containing five samples (30 g) from the same location and sampling year. This process is detailed in Fig. S1. The composite dietary samples were then stored in glass bottles at –30 °C. The study protocol was approved by the Ethics Committee of Kyoto University (Kyoto, Japan). Written informed consent was obtained from all study participants.

2.3. Extraction of the composite dietary samples

Each of the composite dietary samples was subjected to an ion-pair extraction. Briefly, approximately 1 g of each composite dietary sample and an internal standard mixture (100 pg of each $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA, and $^{13}C_2$-labeled PFDoDA) were placed in a 15 mL polypropylene centrifugation tube. Next, 1 mL of 0.5 mol L^{-1} tetrabutylammonium/0.25 mol/L sodium carbonate buffer (pH adjusted to 10 using NaOH) and 1 mL of MTBE were added to the samples, and the tubes were vortex mixed for 60 s. The samples were then centrifuged at 9840 ×*g* for 5 min. The organic layer was separated twice and placed in a clean glass tube, then dried under a gentle stream of nitrogen. The residue was

redissolved in 100 μL of 0.1 mol L^{-1} benzyl bromide/acetone solution containing 1 ng of 11H-perfluoroundecanoic acid and 1 ng of $^{13}C_{12}$-labeled CB111 to monitor the derivatization efficiency. The solution was then derivatized at 60 °C for 1 h. No further clean-up was conducted. Derivatized samples were analyzed by GC/MS within 24 h.

2.4. Instruments and quantification

Derivatized PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA and perfluorotetradecanoic acid (PFTeDA) were analyzed by GC/MS in scan mode with selected ion monitoring (Agilent 6890GC/5973MSD inert, Agilent Technologies Japan, Ltd., Tokyo, Japan). PFCAs were dissolved in 100 μL of 0.1 mol L^{-1} benzyl bromide/acetone solution and derivatized at 60 °C for 60 min with 1 ng of 11H-perfluoroundecanoic acid and 1 ng of $^{13}C_{12}$-labeled CB111 to monitor the derivatization efficiency. After benzylation, the stability was investigated by monitoring the peak area ratio over 24 h. PFCA benzyl esters were separated on a DB-5MS column (30 m length, 0.25 mm i.d., 1 μm film thickness; Agilent Technologies Japan, Ltd.) with a helium carrier gas (99.9999 % purity; Air Liquide Japan Ltd., Tokyo, Japan). Splitless injections (2 μL) were performed with an injector temperature of 220 °C, and the split vent was opened after 1.5 min. The initial oven temperature was 70 °C for 2 min, after which it was increased to 100 C° at 20 °C min^{-1}, and then to 280 °C at 30 °C min^{-1}. To compare the limits of detection, both ECNI and EI were used to quantify the PFCA benzyl esters. In ECNI, methane (99.9999 % purity; Air Liquide Japan Ltd.) was used as the reagent gas (2 mL min^{-1}). The ion source temperature was maintained at 150 °C. In EI, the ion source temperature was maintained at 250 °C. The target ions for

147 determination of PFCAs in both ECNI and EI are summarized in Table 1.

148

149 Standard stock solutions (2 μg mL^{-1}) were diluted to seven working standard solutions
150 (4, 2, 1, 0.8, 0.4, 0.2, and 0.1 ng mL^{-1}) by serial dilution with acetone. All the standard
151 solutions were stored in a refrigerator at 4±2 °C for a maximum period of 3 months
152 from the date of preparation. Quantification was conducted using an internal standard
153 dissolved in acetone. $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA,
154 $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled PFDoDA were used as internal standards for the
155 PFCAs. These standards were diluted to 1 ng mL^{-1}. The instrumental detection limit
156 (IDL) is defined as the mass of the analyte producing a peak with a signal-to-noise ratio
157 of three. Because blank levels were larger for shorter-chain PFCAs than for
158 longer-chain PFCAs, the final net IDLs of the shorter- and longer-chain PFCAs were
159 nearly equivalent.

160

161 **2.5. Method detection limits (MDLs), blank contamination, total extraction**
162 **recovery, and possible matrix effect.**

163 Milli-Q water (Millipore, Billerica, MA) was used as the procedural blank control, and
164 was analyzed after every 10 samples (*n*=6). The procedural blank was extracted using
165 the process described above, and six replicate procedural blanks were prepared
166 independently. In this study, we observed blank contamination for all PFCAs (Table 1).
167 The MDL is defined as the concentration that produces a signal three times that of the
168 blank. The mean blank signal was subtracted from the calculated sample concentration
169 (Table 1). The recoveries of the PFCAs were examined by spiking 100 pg of each
170 standard compound into 10 composite dietary samples before extraction. Possible

matrix effects on the GC-MS detector response were evaluated by comparing the response factors of PFCA benzyl esters in acetone and in food extracts prepared in acetone. The ^{13}C-labeled internal standards (100 pg) were derivatized in acetone before being used for spiking, and then added to the food extracts.

2.6. Statistical analysis

All statistical analyses were conducted using SPSS (Version 16.0 for Windows 2007, IBM Corporation, Armonk, NY). Values of $p<0.05$ were considered statistically significant. Concentrations lower than the detection limits were given a value of half the detection limit for statistical analyses. Statistical analyses were conducted after logarithmic transformation of the PFCAs concentrations. When the statistical tests by two-way ANOVA were significant, analysis of covariance was used to demonstrate the effect of time or location and their interactions influenced on the PFCA levels in the food composite samples.

3. Results and discussion

3.1. Quantification and quality assurance

Derivatization, mass spectra, and IDL. In the present study, we developed a very simple yet sensitive method for PFCAs analysis in food using GC-ECNI-MS with benzyl esterification. Benzyl esterification was used for PFCAs analysis because stabilization of the benzyl radical leaving group preferentially gives carboxylic anions of PFCAs in ECNI.[15] The PFCAs extracted from the composite dietary samples were dissolved in benzyl bromide/acetone solution and derivatized with

11H-perfluoroundecanoic acid and $^{13}C_{12}$-CB-111 (Section 2.3). Peak area ratios to $^{13}C_{12}$-CB-111 showed that the derivatization reaction time of 60 min at 60 °C was sufficient for benzylation to reach completion. After benzylation, the peak area ratios did not change significantly over 24 h (arithmetic mean +/– relative standard deviation: 104±5.6 %, *n*=10).

Mass spectra of the standard solutions were initially acquired in full-scan mode with EI or ECNI to determine the retention times and fragmentation patterns. In EI mode, the molecular ion $[M]^+$ of PFOA benzyl ester was observed at *m*/*z* 504 (Fig. 1). In ECNI mode the PFOA benzyl ester showed an abundant fragment ion $[M\text{-}C_7H_7]^-$ at *m*/*z* 413 (Fig. 1), corresponding to loss of a carboxylate anion ($C_8F_{15}COO^-$). Similar fragmentation was observed among the PFCA benzyl esters with different chain lengths (ECNI mode, Fig. S2; EI mode, Fig. S3). As shown in Table 1, other PFCA benzyl esters also gave abundant fragment ions $[M\text{-}C_7H_7]^-$ in ECNI mode, and the IDLs ranged from 0.003–0.007 pg. Therefore, ENCI was used for quantification of PFCA benzyl esters in subsequent experiments.

Extraction. To avoid freeze-drying the samples and to simplify the method, the PFCAs from the composite dietary samples were extracted by ion-pair extraction[15] into an organic solvent (MTBE). This method prevented co-extraction of water from the food sample.[7] The presence (or absence) of matrix effects on the GC-MS detector response was examined by spiking the food sample extracts with derivatized internal standards immediately before injection into the GC-MS (Section 2.5). The food extracts displayed only minor suppression of the internal standards (95–89 % of solvent based

219 standards). (Table 1) Because no large interferences were observed in the chromatogram
220 in ECNI mode (Table of Contents Art), sample clean-up was not performed after
221 ion-pair extraction.
222
223 **Blank contamination and MDLs**. A widely reported problem in ultra-trace analysis
224 of PFCAs is contamination of procedural blanks.[7, 16] To overcome this problem, all
225 disposable laboratory equipment was sonicated in methanol, and the methanol was
226 analyzed to determine potential sources of contamination. None of the laboratory
227 equipment contributed to background levels of any analyte. The purity of each solvent
228 was tested by evaporating 10 mL of the solvent to dryness. Artifacts originating from
229 the evaporation procedure were investigated by comparing drying the solvent under
230 nitrogen gas and vacuum. Comparable results were obtained with both methods. When
231 the solvents were tested, HPLC-grade MTBE was found to contain low levels of target
232 analytes. Therefore, the HPLC-grade MTBE was distilled by rotary evaporation to
233 reduce the levels of contaminants. The final procedural blank levels were 5 pg g^{-1} for
234 PFOA, 2 pg g^{-1} for PFNA, 1 pg g^{-1} for PFDA, 1.5 pg g^{-1} for PFUnDA, and 1 pg g^{-1} for
235 PFDoDA, PFTrDA and PFTeDA.
236 The mean blank signal was subtracted from the calculated sample concentration only
237 if the calculated sample concentration was three times higher than the blank
238 concentration (Section 2.5). Using this method, we established that the MDLs for
239 PFCAs ranged from 2–10 pg g^{-1} (Table 1). This was one or two orders of magnitude
240 higher than the detection response (lower detection limit) for PFCAs in previous studies
241 (MDLs = 100 pg g^{-1} for PFNA, and 500 pg g^{-1} for PFDA and PFUnDA).[6, 16]
242

Total method recovery. The mean recoveries (n=6, ±relative standard deviation) of the PFCAs obtained by spiking 100 pg of each standard compound into 10 of the composite dietary samples before extraction (Section 2.5) were as follows: 97±16 %, PFOA; 98±19 %, PFNA; 91±17 %, PFDA; 94±18 %, PFUnDA; 90±18 %, PFDoDA; 93±16 %, PFTrDA; and 97±17 %, PFTeDA (Table 1).

Comparison with other methods. There have been fewer reports on analytical methods for PFCAs in composite dietary samples than in serum samples. The methods from two reports for PFCAs are summarized in Table S1.[6, 7] In one of these methods (No. 2 in Table S1), the samples were freeze-dried for pretreatment, and weak anion exchange and dispersive carbon methods were used for subsequent clean-up.[6] Even after purification, the method had a high detection limit (>100 pg g^{-1}) because of the complex sample matrix. In the other method (No. 3 in Table S1), a solid phase extraction column containing florisil and ECNI-carb was used for sample clean-up.[7] This method eliminated matrix effects, and the target compounds were detected at low parts per trillion levels. In our method (No. 1 in Table S1), ion-pair extraction and benzyl esterification were used for sample pretreatment. With ECNI, the detection limits with the present method were comparable to those with one of the earlier methods (No. 3).

3.2. Profile of PFCAs in food composite samples.

The method was applied to 24-h dietary samples from subjects in Japan (Hokkaido, Kyoto, and Okinawa; 1992 and 2009), Korea (Seoul; 1994 and 2007), and China (Beijing; 1993 and 2009). The dietary intakes of PFCAs (nanograms per day) are summarized in Table 2 and Table S2. The levels of the PFCAs were between 39 ng day^{-1}

and 169 ng day^{-1} (per kilogram of body weight) in Korea, 58 ng day^{-1} and 71 ng day^{-1} in China, and 56 ng day^{-1} and 67 ng day^{-1} in Japan. (Table 2) Between the two sampling years, the total levels of PFCAs (C8 to C14) increased significantly (p<0.05). The interaction between the sampling location in Korea and year was significant (p<0.05) (Table 3). The PFCAs with longer chains than PFOA comprised 68 % (1990s) and 82 % (2000s) of the average total PFCAs for the three countries. This finding suggests that the East Asian population has been exposed to both PFOA and long-chain PFCAs.

PFCA exposure from food is only one exposure pathway. Other pathways include exposure to aerosols and household dust. In this study, we estimated the contribution of PFOA from food composite samples in Kyoto. It has been estimated that the adult intake of indoor dust is 50 mg day^{-1}[17] and adult humans inspire 13.3 m^3 of air day^{-1}, with 69 % of the particles in air respirable and PFOAs completely absorbed into the body. The estimated exposure through food composite samples was dominant (86 % of total intake), followed by ambient air (2.4 %) and indoor dust (1.1 %) (Table S3). This result suggests food and drinking water are the major sources of PFOA exposure for humans. However, one earlier study showed the contribution from ambient air was dominant (about 70 % for serum PFOA levels) in Osaka, Japan were there was a strong point source of PFOA [18].

Dietary intakes of PFCAs in the present study (Japan, Korea, and China) are compared with reported data (Japan, Norway) in Table 4. The dietary intakes observed in our study are compared with those from a report on PFCAs (C8–C12) in Norway.[19] In Norway, the dietary intakes (in ng day^{-1}) of long-chain PFCAs from food were 31 for PFOA, 9.5 for PFNA, 13 for PFDA, 6.7 for PFUnDA, and 6.7 for PFDoDA. In contrast

to what was observed in the Japanese and Korean samples, odd-numbered PFCAs did not predominate in the Norwegian samples. This trend is consistent with previous biomonitoring of human serum samples[17,20] which also implies that intake of PFCAs via food may be an important exposure route.

The sources of long-chain PFCAs are not well characterized. A review indicated that odd-numbered PFCAs have been manufactured via oxidation of fluorotelomer olefins[3]. Industrial application of these odd-numbered PFCAs might contribute to the East Asian-specific pattern of PFCAs in daily duplicate diet samples and serum. The temporal increase in long-chain PFCAs, especially in Korea, warrants further investigation of the sources and exposure routes. This would assist in predicting future changes in the food, water, and serum levels of these contaminants.

Even though long-chain PFCAs are prevalent, comprising 82 % of the total PFCAs in this study, their toxicokinetics and toxicities are not well characterized. In several in vitro studies, long-chain PFCAs have caused biological responses at lower doses than PFOA.[21, 22, 23] Because of these uncertainties, comprehensive toxicological studies on long-chain PFCAs are required.

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315 authors have no conflicts of interest to declare.

316

317 **Figure legends**

318 Fig. 1 Mass spectra of PFOA benzyl ester in EI mode and ECNI mode (*m*/*z* 30–800)

319 Fig. S1 Sample collection and treatment

320 Fig. S2 Mass spectra of PFCA benzyl esters in ECNI mode (*m/z* 30–800)

321 Fig. S3 Mass spectra of PFCA benzyl esters in EI mode (*m/z* 30–800)

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References

1. Houde, M.; Martin, J. W.; Letcher, R. J.; Solomon, K. R.; Muir, D. C. G., Biological monitoring of polyfluoroalkyl substances: A review. *Environmental Science & Technology* **2006,** 40, (11), 3463-3473.

2. Renner, R., Scotchgard scotched - Following the fabric protector's slippery trail to a new class of pollutant. In *Sci Am*, 2001; Vol. 284.

3. Prevedouros, K.; Cousins, I. T.; Buck, R. C.; Korzeniowski, S. H., Sources, fate and transport of perfluorocarboxylates. *Environmental Science & Technology* **2006,** 40, (1), 32-44.

4. Harada, K. H.; Hitomi, T.; Niisoe, T.; Takenaka, K.; Kamiyama, S.; Watanabe, T.; Moon, C. S.; Yang, H. R.; Hung, N. N.; Koizumi, A., Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam. *Environ Int* **2011,** in press.

5. D'Hollander, W.; de Voogt, P.; De Coen, W.; Bervoets, L., Perfluorinated Substances in Human Food and Other Sources of Human Exposure. *Reviews of Environmental Contamination and Toxicology, Vol 208* **2010,** 208, 179-215.

6. Karrman, A.; Harada, K. H.; Inoue, K.; Takasuga, T.; Ohi, E.; Koizumi, A., Relationship between dietary exposure and serum perfluorochemical (PFC) levels-A case study. *Environment International* **2009,** 35, (4), 712-717.

7. Vestergren, R.; Ullah, S.; Cousins, I. T.; Berger, U., A matrix effect-free method for reliable quantification of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids at low parts per trillion levels in dietary samples. *Journal of Chromatography A* **2012,** 1237, 64-71.

8. Scott, B. F.; Moody, C. A.; Spencer, C.; Small, J. M.; Muir, D. C. G.; Mabury, S. A., Analysis for perfluorocarboxylic acids/anions in surface waters and precipitation using GC-MS and analysis of PFOA from large-volume samples. *Environmental Science & Technology* **2006,** 40, (20), 6405-6410.

9. De Silva, A. O.; Mabury, S. A., Isomer distribution of perfluorocarboxylates in human blood: Potential correlation to source. *Environmental Science & Technology* **2006,** 40, (9), 2903-2909.

10. Koizumi, A.; Harada, K. H.; Inoue, K.; Hitomi, T.; Yang, H. R.; Moon, C. S.; Wang, P.; Hung, N. N.; Watanabe, T.; Shimbo, S.; Ikeda, M., Past, present, and future of environmental specimen banks. *Environ Health Prev Med* **2009,** 14, (6), 307-18.

11. Koizumi, A.; Yoshinaga, T.; Harada, K.; Inoue, K.; Morikawa, A.; Muroi, J.; Inoue, S.; Eslami, B.; Fujii, S.; Fujimine, Y.; Hachiya, N.; Koda, S.; Kusaka, Y.; Murata, K.; Nakatsuka, H.; Omae, K.; Saito, N.; Shimbo, S.; Takenaka, K.; Takeshita, T.; Todoriki, H.;

Wada, Y.; Watanabe, T.; Ikeda, M., Assessment of human exposure to polychlorinated biphenyls and polybrominated diphenyl ethers in Japan using archived samples from the early 1980s and mid-1990s. *Environmental Research* **2005,** 99, (1), 31-39.

12. Ikeda, M.; Zhang, Z. W.; Shimbo, S.; Watanabe, T.; Nakatsuka, H.; Moon, C. S.; Matsuda-Inoguchi, N.; Higashikawa, K., Exposure of women in general populations to lead via food and air in east and southeast Asia. *American Journal of Industrial Medicine* **2000,** 38, (3), 271-280.

13. Desalegn, B.; Takasuga, T.; Harada, K. H.; Hitomi, T.; Fujii, Y.; Yang, H. R.; Wang, P. Y.; Senevirathna, S. T. M. L. D.; Koizumi, A., Historical trends in human dietary intakes of endosulfan and toxaphene in China, Korea and Japan. *Chemosphere* **2011,** 83, (10), 1398-1405.

14. Harada, K. H.; Takasuga, T.; Hitomi, T.; Wang, P. Y.; Matsukami, H.; Koizumi, A., Dietary Exposure to Short-Chain Chlorinated Paraffins Has Increased in Beijing, China. *Environmental Science & Technology* **2011,** 45, (16), 7019-7027.

15. Galdiga, C. U.; Greibrokk, T., Ultra trace determination of fluorinated aromatic carboxylic acids in aqueous reservoir fluids by solid phase extraction in combination with negative ion chemical ionisation mass spectrometry after derivatisation with pentafluorobenzyl bromide. *Fresenius Journal of Analytical Chemistry* **1998,** 361, (8), 797-802.

16. Fujii, Y.; Yan, J. X.; Harada, K. H.; Hitomi, T.; Yang, H.; Wang, P. Y.; Koizumi, A., Levels and profiles of long-chain perfluorinated carboxylic acids in human breast milk and infant formulas in East Asia. *Chemosphere* **2012,** 86, (3), 315-321.

17. Aung, N. N.; Yoshinaga, J.; Takahashi, J., Exposure assessment of lead among Japanese children. *Environ Health Prev Med* **2004,** 9, (6), 257-61.

18. Niisoe, T.; Harada, K. H.; Ishikawa, H.; Koizumi, A., Long-Term Simulation of Human Exposure to Atmospheric Perfluorooctanoic Acid (PFOA) and Perfluorooctanoate (PFO) in the Osaka Urban Area, Japan. *Environmental Science & Technology* **2010,** 44, (20), 7852-7857.

19. Haug, L. S.; Salihovic, S.; Jogsten, I. E.; Thomsen, C.; van Bavel, B.; Lindstrom, G.; Becher, G., Levels in food and beverages and daily intake of perfluorinated compounds in Norway. *Chemosphere* **2010,** 80, (10), 1137-1143.

20. Haug, L. S.; Thomsen, C.; Bechert, G., Time Trends and the Influence of Age and Gender on Serum Concentrations of Perfluorinated Compounds in Archived Human Samples. *Environmental Science & Technology* **2009,** 43, (6), 2131-2136.

21. Liao, C. Y.; Wang, T.; Cui, L.; Zhou, Q. F.; Duan, S. M.; Jiang, G. B., Changes in Synaptic Transmission, Calcium Current, and Neurite Growth by Perfluorinated

Compounds Are Dependent on the Chain Length and Functional Group. *Environmental Science & Technology* **2009,** 43, (6), 2099-2104.

22. Matsubara, E.; Harada, K.; Inoue, K.; Koizumi, A., Effects of perfluorinated amphiphiles on backward swimming in Paramecium caudatum. *Biochemical and Biophysical Research Communications* **2006,** 339, (2), 554-561.

23. Upham, B. L.; Deocampo, N. D.; Wurl, B.; Trosko, J. E., Inhibition of gap junctional intercellular communication by perfluorinated fatty acids is dependent on the chain length of the fluorinated tail. *International Journal of Cancer* **1998,** 78, (4), 491-495.

Table 1
Quality assurance for PFCAs analysis in food samples

Compound	(carbon atoms)	Quantification (confirmation) ECNI	Quantification (confirmation) EI	Instrument detection limit [a] (pg) ECNI	Instrument detection limit [a] (pg) EI	Recovery and (reproducibility) % (RSD%)[b] (n=10, fortified)[c]	Relative detector response[d] % (SD%) (n=6, fortified)[d]	Procedural blank (SD) pg g^{-1}, n=6,	Method detection limit[e] pg g^{-1}
PFOA	(C8)	413 (414)	504 (485)	0.003	0.2	97 (16)	95(2.8)	5(0.4)	10
PFNA	(C9)	463 (464)	554 (535)	0.003	0.2	98 (19)	93(3.9)	2(0.3)	4
PFDA	(C10)	513 (514)	604 (585)	0.004	0.2	91 (17)	94(4.9)	1(0.3)	2
PFUnDA	(C11)	563 (564)	654 (635)	0.004	0.2	94 (18)	92(6.3)	1.5(0.4)	3
PFDoDA	(C12)	613 (614)	704 (685)	0.005	0.4	90 (18)	89(7.7)	1(0.2)	2
PFTrDA	(C13)	663 (664)	754 (735)	0.005	0.4	93 (16)	-	1(0.2)	2
PFTeDA	(C14)	713 (714)	804(785)	0.007	2	93 (17)	-	1(0.4)	2

[a] 1 µL injection

[b] RSD: relative standard deviation

[c] The recoveries of the PFCAs were examined by spiking 100 pg of each standard compound into 10 composite dietary samples before extraction.

[d] $^{13}C_4$-labeled PFOA, $^{13}C_5$-labeled PFNA, $^{13}C_2$-labeled PFDA, $^{13}C_2$-labeled PFUnDA and $^{13}C_2$-labeled PFDoDA were derivatized and were fortified at 100 pg to food extracts. Matrix effects are expressed as relative detector responses (%) to the signal area responses of corresponding solvent-based preparations.

[e] food sample of 1 g. The method detection limit is defined as the concentration that produces a signal three times that of the blank. The mean blank signal was subtracted from the calculated sample concentration.

Table 2

Dietary intake of PFCAs from composite food samples (ng day^{-1})

		Year (No. of pooled diets)		ng day^{-1} PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	Total (C8-C14)
China	Beijing	1993	n>MDL (%)	0(0)	3(60)	4(80)	4(80)	4(80)	4(80)	2(40)	4(80)
			Median (Range)	<22.5	9.4(n.d.-12.3)	8.9(n.d.-15.4)	9.6(n.d.-13.9)	6.5(n.d.-13.3)	15.0(n.d.-16.0)	<4.5	66.6(n.d.-80.2)
		(n=5)	Mean±SD	-	<9.0	9.0±4.9	9.7±4.0	7.3±4.1	12.6±5.8	-	61.7±20.0
			GM (GSD)	-	<9.0	7.6(2.1)	8.8(1.7)	6.3(1.9)	10.3(2.4)	-	58.1(1.5)
		2009	n>MDL (%)	0(0)	2(40)	5(100)	2(40)	3(60)	3(60)	2(40)	5(100)
			Median (Range)	<30.9	<12.4(n.d.-15.8)	13.8(6.9-19.1)	<9.3(n.d.-32.4)	8.7(n.d.-14.9)	8.0(n.d.-29.6)	<6.2(n.d.-19.4)	68.1(35.1-141.8)
		(n=5)	Mean±SD	-	9.4±4.9	13.1±4.9	-	8.0±5.3	13.0±12.1	-	78.7± 40.8
			GM (GSD)	-	8.4(1.7)	12.2(1.5)	-	6.4(2.2)	8.4(3.0)	-	70.7(1.7)
Korea	Seoul	1994	n>MDL (%)	0(0)	0(0)	2(40)	4(80)	2(40)	5(100)	1(20)	5(100)
			Median (Range)	<17.8	<7.7	<3.6(n.d.-6.8)	8.2(n.d.-13.2)	<3.6(n.d.-5.2)	9.3(5.2-22.2)	<3.6(n.d.-3.7)	40.4(28.8-56.4)
		(n=5)	Mean±SD	-	-	-	8.5±4.4	-	10.5±6.9	-	40.1±11.6
			GM (GSD)	-	-	-	7.3(1.9)	-	9.0(1.8)	-	38.8(1.3)
		2007	n>MDL (%)	0(0)	2(40)	5(100)	5(100)	5(100)	5(100)	5(100)	5(100)
			Median (Range)	<21.0	<8.4(n.d.-16.1)	8.6(6.8-13.4)	60.3(46.9-80.2)	17.1(12.6-25.3)	49.3(41.4-67.8)	9.6(5.6-11.4)	172.8(132.3-225.2)
		(n=5)	Mean±SD	-	-	9.4±2.8	63.4±12.4	17.4±4.9	54.1±11.1	9.4± 2.3	171.6±34.8
			GM (GSD)	-	-	9.1(1.3)	62.4(1.2)	16.9(1.3)	53.2(1.2)	9.2(1.3)	168.9(1.2)
Japan	Hokkaido	1992, 1995	n>MDL (%)	3(43)	1(14)	2(29)	7(100)	2(29)	7(100)	0(0)	7(100)
			Median (Range)	<22.2(n.d.-35.8)	<8.9(n.d.-13.7)	<4.4(n.d.-5.2)	14.5(8.9-25.0)	<4.4(n.d.-4.9)	13.1(5.2-29.7)	<4.4	79.3(33.8-88.5)
		(n=7)	Mean±SD	-	-	-	15.3±4.8	-	15.7±9.8	-	64.6±22.4
			GM (GSD)	-	-	-	14.7(1.4)	-	13.2(1.9)	-	60.8(1.5)
		2009	n>MDL (%)	2(29)	4(57)	3(43)	7(100)	5(71)	6(86)	3(43)	7(100)
			Median (Range)	<18.1(n.d.-25.4)	7.8(n.d.-20.3)	<3.6(n.d.-11.3)	20.6(14.7-30.0)	4.9(n.d.-16.1)	14.5(n.d.-40.0)	<3.6(n.d.-9.4)	57.8(50.7-146.8)
		(n=7)	Mean±SD	-	8.6±6.1	-	22.3±5.4	6.0±4.8	18.4±12.8	-	76.5± 37.2
			GM (GSD)	-	<7.6	-	21.7(1.3)	4.7(2.0)	13.4(2.7)	-	70.5(1.5)
	Kyoto	1996, 1997	n>MDL (%)	6(100)	1(17)	1(17)	5(83)	1(17)	5(83)	0(0)	6(100)
			Median (Range)	23.7(19.7-30.6)	<7.5	2.0(n.d.-4.5)	8.7(n.d.-17.2)	<3.7(n.d.-4.3)	5.6(n.d.-13.6)	<3.7	46.7(38.4-79.2)
		(n=6)	Mean±SD	24.3±3.6	-	-	9.1±4.6	-	6.2±4.0	-	49.8±15.1
			GM (GSD)	24.1(1.2)	-	-	8.1(1.7)	-	5.3(1.9)	-	48.3(1.3)
		2009	n>MDL (%)	5(83)	3(50)	2(33)	4(67)	2(33)	5(83)	1(17)	6(100)
			Median (Range)	23.9(n.d.-38.2)	<6.3(n.d.-9.9)	<3.1(n.d.-5.9)	8.4(n.d.-33.0)	<3.1(n.d.-9.3)	9.7(n.d.-34.8)	<3.1(n.d.-4.3)	56.6(23.5-117.5)
		(n=6)	Mean±SD	24.0±9.9	-	-	11.4±11.5	-	12.5±11.8	-	61.6±34.2
			GM (GSD)	21.8(1.7)	-	-	7.4(2.8)	-	8.4(2.9)	-	53.8(1.8)
	Okinawa	1992, 1995	n>MDL (%)	3(43)	0(0)	1(14)	6(86)	0(0)	6(86)	0(0)	6(86)
			Median (Range)	<25.9(n.d.-49.2)	<10.4	<5.2	14.4(n.d.-21.0)	<5.2	10.6(n.d.-16.2)	<5.2	55.5(n.d.-93.9)
		(n=7)	Mean±SD	-	-	-	13.5±5.9	-	10.6±4.9	-	62.3±25.9
			GM (GSD)	-	-	-	11.9(1.9)	-	9.1(2.0)	-	57.4(1.6)
		2009	n>MDL (%)	4(57)	6(86)	6(86)	7(100)	6(86)	7(100)	3(43)	7(100)
			Median (Range)	19.2(n.d.-26.6)	9.0(n.d.-11.9)	4.0(n.d.-8.2)	20.7(12.0-30.6)	5.1(n.d.-10.1)	15.9(8.0-26.0)	<3.7(n.d.-8.1)	86.8(52.1-92.2)
		(n=7)	Mean±SD	<18.5	9.0±2.6	4.8±2.2	20.6±6.9	5.7±2.7	16.5± 5.4	-	78.0±16.2
			GM (GSD)	<18.4	8.6(1.4)	4.4(1.6)	19.6(1.4)	5.1(1.8)	15.7(1.4)	-	76.4(1.3)

N.D.: not detected; MDL: method detection limit; SD: standard deviation; GM: geometric mean; GSD: geometric standard deviation.

Concentrations lower than the detection limits were given a value of half the detection limit for statistical analyses.

Table 3
Statistical tests for PFCAs in food composite samples

a. Two-way analysis of variance

Factor	Locations	Year	Interaction (location*year)
p-value	0.292	**0.003***	**0.002***

b. Analysis of covariance with PFCAs food intakes, historical and demographic status in East Asia.

	A model for PFCAs[a]									
	Intercept	Locations (Kyoto=0)				Year (2000s=1)	Interaction (location*Year (2000s=1))			
		Hokkaido	Okinawa	Beijing	Seoul		Hokkaido	Okinawa	Beijing	Seoul
		α	β	γ	σ	ε	ζ	η	θ	ι
Parameter e	1.822	0.007	-0.072	-0.012	0.098	0.077	-0.020	-0.084	-0.027	0.223
t-value	-	0.150	-1.580	-0.230	1.910	3.170	-0.430	-1.850	-0.520	4.350
p-value	-	0.880	0.120	0.821	0.062	**0.003***	0.668	0.070	0.606	**<.0001***

[a] Regression models are described as (log PFCAs food intakes)=(intercept) + αx [location:Hokkaido] + βx [location:Okinawa] + γx [location:Beijing] + σx [location:Seoul] + εx [Year] + ζx [interaction:Hokkaido] + ηx [interaction:Okinawa] + θx [interaction:Beijing] + ιx [interaction:Seoul]

* indicates significant difference ($p<0.05$)

Table 4
Comparison of dietary intakes of PFCAs observed in the present study (Japan, Korea, China) with reported data (Japan, Norway)

Sampling site	Year	Study type		Dietary intake (ng day^{-1}) PFOA (C8)	PFNA (C9)	PFDA (C10)	PFUnDA (C11)	PFDoDA (C12)	PFTrDA (C13)	PFTeDA (C14)	reference
Japan											
Overall Japan	1990s	daily duplicate diet	Mean	22.8	<8.9	<4.4	12.8	<4.4	11.1	<4.4	This study
	2009	daily duplicate diet	Mean	18.0	7.9	3.9	18.4	<3.6	16.0	<3.6	This study
Hokkaido	1992, 1995	daily duplicate diet	Mean	<22.5	<9.0	<4.5	15.3	<4.5	15.7	<4.5	This study
	2009	daily duplicate diet	Mean	<19.0	8.6	3.9	22.3	6.0	18.4	4.0	This study
Kyoto	1996, 1997	daily duplicate diet	Mean	24.3	<7.0	<3.5	9.1	<3.5	6.2	<3.5	This study
	2009	daily duplicate diet	Mean	24.0	<6.3	<3.2	11.4	<3.2	12.5	<3.2	This study
Okinawa	1992, 1995	daily duplicate diet	Mean	<26.1	<10.5	<5.2	13.5	<5.2	10.6	<5.2	This study
	2009	daily duplicate diet	Mean	<18.5	9.0	4.8	20.6	5.7	16.5	3.8	This study
Osaka	2004	daily duplicate diet	Mean	68.5	-	-	-	-	-	-	Karrman et al., 2009
Miyagi	2004	daily duplicate diet	Mean	48.6	-	-	-	-	-	-	Karrman et al., 2009
Korea											
Seoul	1994	daily duplicate diet	Mean	<17.8	<7.1	<3.6	8.5	<3.6	10.5	<3.6	This study
	2007	daily duplicate diet	Mean	<20.6	<8.2	9.4	63.4	17.4	54.1	9.4	This study
China											
Beijing	1993	daily duplicate diet	Mean	<22.5	<9.0	9.0	9.7	7.3	12.6	<4.5	This study
	2009	daily duplicate diet	Mean	<30.5	<12.2	13.1	11.0	8.0	13.0	9.0	This study
Norway	2008-2009	estimated intakes	Mean	31	9.5	13	6.7	6.7	-	-	Haug et al., 2010

a Calculated assuming a body weight of 70 kg

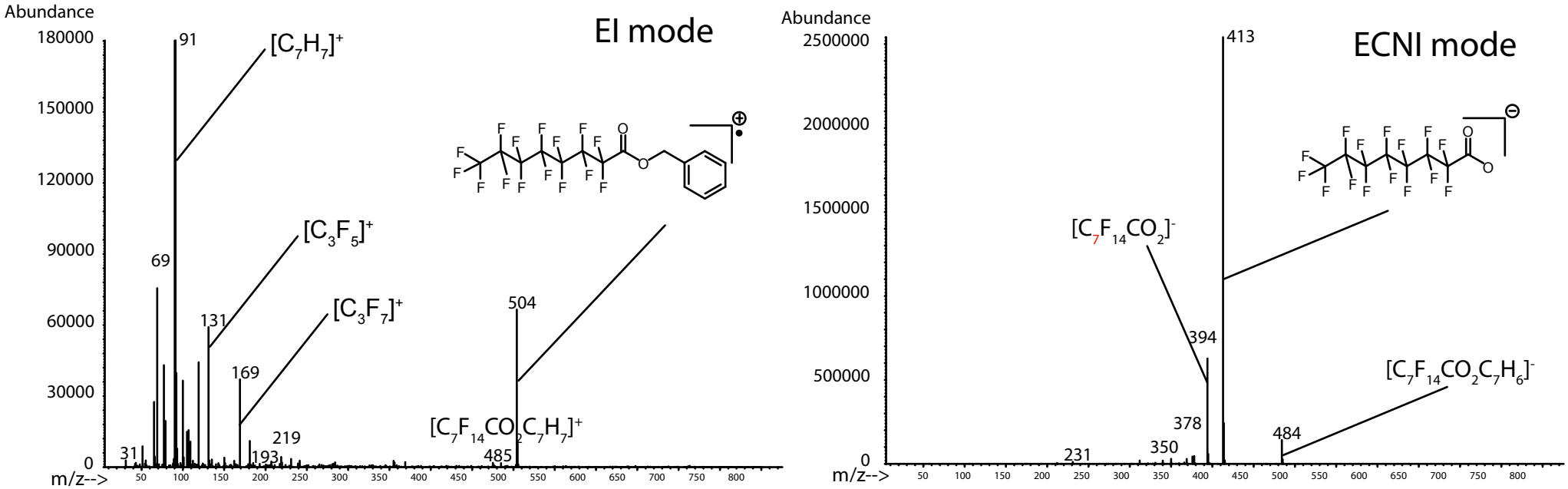

Fig. 1 Mass spectra of PFOA benzyl ester in EI mode and ECNI mode (m/z 30−800)